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The Effect of Chemicals on Weed and Conifer Seedlings

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THE EFFECT OF CHEMICALS

ON

WEED AND CONIFER SEEDLINGS

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INTRODUCTION

As reforestation becomes more important in the United States, there is an increasing demand for forest tree seedlings. The production of the seedlings is a somewhat specialized phase of the industry calling into use certain details of cultural practice not employed elsewhere. One of the major items of expense in the care of forest nursery seed beds is the control of weeds. Wakeley (8) estimates that the total cost of hand-weeding untreated seed beds may be reduced two-thirds to four-fifths by the use of chemicals. It is apparent that any procedure which will reduce the weeds will materially reduce the cost of tree seedlings and may encourage reforestation in proportion.

The use of chemicals as a means of controlling weeds in forest nurseries has not been generally accepted by nurserymen, probably because of the lack of definite information regarding environmental factors at their particular nurseries. Wahlenberg* suggests that due to the variable factors of soil, temperature, moisture, etc., the results secured by the use of a certain method at one nursery may vary widely from the results obtained by the use of the same method at another nursery. By use of the technique worked out by other investigators, the writer has attempted to add to the information available on chemical weed control.

*Manuscript by W. G. Wahlenberg. July, 1926.

REVIEW OF LITERATURE

Wahlenberg (7) carried on extensive experiments with Western White Pine, Western Yellow Pine and Engelmann Spruce at the Savenac nursery in western Montana. The treatment he used consisted of applying to every square foot of seed-bed area, 8 grams of zinc sulphate dissolved in 250 c.c. of water. The application was made immediately after the seed was sown. Results varied from year to year, but each trial effected a reduction of from 80 to 100 per cent in the number of weeds present in the seed beds for two seasons following the application. The treatment, however, did not kill advanced growth of weeds nor pieces of roots or underground stems left from hand pulling, but it did prevent the germination of most of the weed seed. The seeds appeared to be killed just after breaking the seed coats in an attempt to germimate. The treatment seemed to be especially fatal to clover seed, and effective on most of the other native weed seeds. In general, the grasses were observed to be least affected. Zinc sulphate did not prevent the use of field peas as a green fertilizer crop--apparently because much of the original dose of zinc sulphate had been leached out of the soil before the pea seed was sown. In fact, in the case of peas grown on treated soil, nodule development and, hence, nitrogen fixation were markedly stimulated. An annual saving in labor cost of weeding, amounting to $353.00, or 32 cents per bed of 48 square feet, was reported from the

Savenac nursery.

Wahlenberg* found that zinc sulphate had a tendency to stimulate the germination of pine seed, not only by reducing the time necessary to complete the germination of all viable seeds, but also by increasing the total number of individuals that sprout. The treatment was found in no way detrimental to the subsequent development, survival, and growth of Western White Pine (Pinus monticola D.Don.) and Western Yellow Pine (Pinus ponderosa Laws.) planting stock. The germination of Engelmann Spruce (Picea Engelmannii Engelm.) was not injured by the treatment, but that of Western Red Cedar (Thuja plicata D.Don.) seemed to be reduced--probably because of the thin seed coats.

Hartley (3) states that one-fourth ounce of zinc sulphate dissolved in one pint of water (when used on wet soil) or one and one-half pints of water (when used on dry soil) applied per square foot of bed two weeks before sowing is used to control "damping-off" of conifers.

Herbert (4) in 1925 tested the action of zinc sulphate on Pinus strobus L. and Picea abies (L.) Karst. Greenhouse flats were filled with sterile sand and seeds of Pinus strobus, Picea abies, and various weed seeds were sown. Zinc sulphate solutions were applied at the rate of one-fourth ounce, one-half ounce, three-fourths ounce, and one ounce per plot. Results: Weeds germinated

*Manuscript by W. G. Wahlenberg. July, 1926.

in all flats although fewer appeared in the flats treated with one ounce of zinc sulphate. There was no "damping-off" present in any of the flats nor was there any marked difference in the germinative energy period or in the germination per cent. In a field test, two seed beds were treated with one-fourth ounce of zinc sulphate per square foot, and two others were treated with one-half ounce per square foot. Sterile sand was used on the beds to prevent "damping-off". Artificial watering was resorted to whenever necessary. Results: No weed growth took place in beds treated with one-half ounce per square foot, but no apparent effect of the chemical was noticeable in the case of the two beds treated with only one-fourth ounce per square foot. Apparently, because of the system of watering, or the soil character, it was necessary to use greater strengths of chemical than are recommended by Wahlenberg.

Hansen* used zinc sulphate in amounts recommended by Wahlenberg and found that it apparently controlled such weeds as grasses, but that it had no effect on buckwheat. It caused no apparent injury to the tree seedlings, nor did it reduce the germination of any of the species tested, which were Norway Pine (Pinus resinosa Ait.), White Spruce (Picea glauca Voss.) and White Pine (Pinus strobus L.)

The zinc-sulphate method of weed control developed at the

*Personal correspondence with T. S. Hansen, Cloquet, Minn.

Savenac nursery by W. G. Wahlenberg was tried out on Longleaf Pine (Pinus palustris Mill.) by the Southern Forest Experiment Station (8). The test was so successful that the station persuaded the Great Southern Lumber Co. to treat a 4 by 160 foot bed of Longleaf Pine in their Bogalusa nursery. The bed was treated with 8 grams of commercial zinc sulphate per square foot. About a month after sowing the seed, counts were made on 20 square feet of a treated bed and 20 square feet of an untreated bed. Germination was higher and the mortality was lower in the treated beds. Weeds other than grass were practically eliminated. Three days later, when the germination was practically complete, the same areas were again examined. The untreated beds than showed 10 per cent fewer seedlings than the treated beds, three times as many grasses, and sixty-two times as many weeds other than grass. The cost of treating the 640 square foot bed was about $1.80. It was estimated that the total cost of eradication of weeds from treated plots was from one-third to one-fifth that of hand-weeding untreated beds of the same size.

The Swedish writer, Juhlin-Hannfelt (5) describes certain experiments made (1925-26) with zinc sulphate for the destruction of weeds in forest nurseries. He worked with a number of weeds and found that 73.1 per cent of all weeds tested were killed by applications of 60 grams of zinc sulphate per square meter dissolved in 2.5 liters of water. Pine seeds were also tested for the influence of the chemical on the germinative faculty of the seeds by placing the seeds on filter paper or in flower pots filled with garden soil, and then adding a 3.6 per cent solution of zinc sulphate dissolved in 2.5 liters of water per square meter. The germination percentage of seeds placed on filter paper was 65.5 and the germination of seeds in flower pots was 51.5.

Sulphuric acid as a preventive of the growth of weeds has been used successfully by Claridge (2) in the State Forest Nursery at the North Carolina State Agricultural College. Three sixteenths of an ounce of commercial sulphuric acid (57 per cent) diluted with one pint of water was applied to each square foot of nursery bed. The seeds (principally of Loblolly, Longleaf, Shortleaf, and Slash Pine) were placed in the seed beds and covered lightly with sand, and the diluted acid was applied immediately by means of a watering can.

Hartley (3), working at Halsey, Nebraska, tried a number of compounds on four groups of plants (Equisetum, pines, grasses, and dicotyledons) found that of these four groups of plants represented, the higher the group in the evolutionary scale, the greater the susceptibility of its representatives to injury, not only to sulphuric acid but to hydrochloric and nitric acids and certain toxic salts such as copper sulphate, ferrous sulphate, mercuric chloride and ammoniacal copper carbonate.

He found that pine seedlings (Pinus banksiana Lamb., Pinus ponderosa Laws., Pinus resinosa Ait., Pinus laricio Poir.) treated with sulphuric acid at or before the time of sowing took the form of damage to the growing apices of the radicles, with the result that the extension of the root was stopped. Whether the meristematic apical cells were actually killed or simply lost their meristem qualities was not determined, though the former was thought more probable. In most cases root apices rendered incapable of growth retained their normal cream color for a few days after the injury and often recovered, though in severe cases they turned dark very soon. Ordinarily the growth of cells just back of the apex was not entirely prevented, so that the root tips became truncated as a result of the uneven growth. In order to distinguish acid injury from injury due to parasites, seedlings were dug up a week or ten days after they emerged and were found to have the following characteristics:

1. Roots of injured seedlings.--Length, one-fourth to five-eighths of an inch. Color, if brown at all, tip will be as brown as the rest. Firm throughout.

2. Roots of healthy seedlings.--Length, one to three inches. Color, upper part may be brown, but tip will be white.

3. Roots of damped-off seedlings (attacked by parasite.--Length, usually same as healthy, but lower part may be entirely decayed, making root appear short. Some parts of roots examined will always be found soft from decay, while acid-injured roots are firm throughout.

Hartley also pointed out that frequent light watering is better than heavier and less frequent applications. He showed that in a single period of 11 hours the moisture content of the surface soil at four different points in the seed beds dropped from 12.02 to 1.85 per cent (in a moisture content determination). This of necessity caused great variations in the concentration of the soil solution. A little below the surface the moisture is more stable. The most rapid loss of moisture found in seed beds, from one to two inches in depth, during the period in which determinations were made, was a drop of from 17 to 11.5 per cent in an interval of approximately 36 hours. This explains the relative safety of roots which have penetrated below the upper half inch of soil. In addition to the increased concentration of the acid solution already in the surface soil (due to the decrease of the solvent) acid from lower levels is presumably brought up to the surface by the capillary rise of the soil solution to replace water lost by evaporation. When the treated soil is soaked thoroughly with water and subjected to continuous evaporation for several days, but at a rate slow enough to avoid drying the surface soil entirely and breaking the capillary connection, this continuous upward movement of solution ultimately produces killing concentrations in the surface soil, even while it is still very moist. The problem of preventing injury to seedlings therefore becomes one not only of keeping the surface soil moist, but of maintaining a fairly constant downward movement of soil moisture, or, at least, of preventing a continuous upward movement for any considerable period, until after the roots of all seedlings have extended half an inch into the soil. Experience has shown that this can be done more easily with frequent light waterings than with heavier and less frequent applications. A watering system worked out by Hartley proved entirely successful in preventing injury to pines from acid applied at the time of sowing. In clear weather, water was applied at the rate of 0.3 of an inch; in cold and cloudy weather, it was applied at the rate of 0.2 of an inch. He watered twice daily when the temperature exceeded 80 degrees F., once daily in ordinary spring weather, and every other day or even less often in misty or rainy weather. No difference was noted in extent of injury to seedlings whether 0.25 of an ounce of acid was dissolved in 64 or in 192 volumes of water.

Hartley found that the root above the tip would resist relatively high concentrations of acids, while the tip of the root was found to be most sensitive to poisons. He quotes McCool, who, working with barium, sodium and other chemicals, found that root tips a few days old are less susceptible to injury than those of seedlings which have just germinated. Hence, as he suggested, the age of the seedling may be more important than location of root tips in making older seedlings more resistant.

Brenchley (1) has reviewed the literature on the effect of zinc on growth of the higher plants. The following is a direct quotation from Ch. IV of her text:

"EFFECT OF ZINC COMPOUNDS OF PLANT GROWTH WHEN THEY ARE PRESENT IN SOILS.--As soon as the presence of zinc in members of the vegetable kingdom was established the question arose as to its effect upon both the plant and the soil.

Gorup-Bezanez (1863) grew plants in soil with which 30 grams of metallic poisons such as $CuSO_4$, $ZnSO_4$, HgO, were intimately mixed with 30.7 litres ('cubik Decimeter') of soil[1]. On analyzing the ash of Secale cereale, Polygonus Fagopyrum, and Pisum sativum after six months growth he failed to detect the presence of zinc in any one of the three. As the results varied with different poisons on different plants he concluded that the absorption capacity of the various kinds of soils for different poisons varies, that basic salts are absorbed, while the acid salts may pass completely through the soil in the drainage water.

Freytag (1868) stated that zinc is retained by the soil in the form of oxide, which is derived from dilute zinc compounds as they filter through the soil, by decomposition by the salts of the soil. For field earth the limit of absorption of zinc oxide sulphate is between .21% - .24% of the earth.

F. C. Phillips (1882) corroborated Freytag's statement as to the absorption of small quantities of zinc by the roots of plants, but he states as a fact that both lead and zinc may enter plant tissues without causing any disturbance in the growth, nutrition or functions of the plants, a conclusion that is obviously incorrect or at least incomplete in view of later work on the subject. His choice of plants was certainly unusual, including geraniums, coleas, ageratums and pansies, the poison used being zinc carbonate.

Holdefleiss (1883) stated that in spite of a soil content of 2% zinc the vegetation was not in any way harmed, clover fields and meadow lands on zinc soil presenting a normal appearance. This observation was quite inconclusive, as the author proceeds to say that of the plants that were able to absorb zinc salts without disadvantage the most luxuriant were the so-called zinc plants--the exceptions that prove the rule. Two years later Baumann showed that such insoluble zinc salts as the carbonate and sulphide in the soil cannot hurt plants. These salts are certainly dissolved to some extent by water containing CO_2 but solution is hindered by the constitution of the soil. He also found that the various kinds of soil act differently upon zinc solutions, the

1.This is equivalent to about .1% of poison.

absorptive power of pure humus soils ('reinem Humusboden') for zinc solutions being the strongest. Clay and chalk soils also decompose such solutions energetically, while poor sandy soils have only a weak power of absorption. This selectivity of absorption may account for the difference in the toxicity of zinc salts to plants in the various soils.

Storp (1883) experimented to determine the changes in the various characters of the soil by the action of zinc salts on it, and he makes the remarkable statement that in some soils the presence of zinc generates free sulphuric acid, which is particularly injurious to plant life. Grasses, young oaks and figs showed a decrease in dry weight, nitrogen and fat, as the quantity of zinc compounds increased in the water added to the soil. Both the quality and the quantity of the crop were adversely affected. This decrease in the dry weight due to the presence of zinc was confirmed by Jensch later on, and also by Nobbe, Baessler and Will (1884), who state that both lead and zinc compounds work disadvantageously to vegetation even when they are present in such small quantities that the plants are outwardly sound, the harmful action appearing in the decrease of dry weight. Contrary to Baumann's opinion, zinc carbonate is said to be one of the salts that exercises this insidious poisonous action. Storp (1883) noticed that the direct poisonous action of zinc compounds is largely destroyed by their admixture with soil, but he suggests that a secondary cause of harm is introduced by the accumulation of insoluble zinc salts, so that the fertility of the soil is impaired to the detriment of the vegetation.

Ehrenberg (1908) throws out a suggestion that zinc is specially harmful to plant life when it occurs in conjunction with ammonia, but no further evidence has come to light.

"MODE OF ACTION OF ZINC ON PLANTS.--The reason for the toxicity of zinc salts when present in soil forced itself upon the attention of some of the early investigators in this field. Freytag (1868) put forward the hypothesis that the zinc oxide is partly or exclusively absorbed by the roots on account of the cell walls of the root being corroded by the very thin layer of zinc salts lying in contact with it--the same theory as has been held with regard to copper. He stated also that the quantity of zinc oxide taken up by the plant through its roots is strictly limited, not being proportional to the quantity occurring in the soil, but varying between narrow limits. Krauch (1882) found himself unable to accept another hypothesis which at one time found favour, i.e. that the zinc salts kill the plants by coagulating the protoplasm. If this were so, he argued, no plants at all could grow upon soils containing zinc, and he was content to leave the causes as one yet to be explained. Even at the present time, thirty years after, we know very little more about the physiological cause of the toxicity of zinc.

"EFFECT OF ZINC COMPOUNDS ON GERMINATION.--In the course of his investigations on the influence of zinc on vegetation Freytag just touched upon the question of seed germination. According to his statement the presence of zinc oxide in the soil does not exercise much influence upon germination and the growth processes of plants. Little zinc is stored up in seeds and on this account seeds originating from plants containing zinc germinate quite normally and do not seem to be affected by the peculiar nutritive conditions of the parent plants.

In certain cases light seems to have something to do with the harm zinc compounds work on plants. Storp found that when clover seeds were germinated in the dark on filter paper moistened with water containing .025 gm. ZnO per litre (added in the form of zinc sulphate) no deleterious action was observed. Barley seeds were soaked for four days in (a) distilled water, (b) water with .9 gm. ZnO per litre, which was frequently changed. These seeds were then placed in the dark on filter papers soaked respectively with water and with the solution containing ZnO. So long as no light was admitted, for a period of eleven days, germination was uniform in both sets, but directly the covers were removed the growth of the seeds with zinc ceased almost entirely, and they did not assume the green colour taken on by the unpoisoned seedlings. With maize the germination was retarded by zinc even in the dark, but the harmful action of light on the plants with zinc was again established. These results seem to indicate that the formation and activity of chlorophyll is impaired by the toxic agent, and this hypothesis is borne out by the fact that in many fungi and non-assimilating higher plants the toxic action of zinc is not evident.

Micheels (1906) approached the matter from a totally different standpoint, seeking to discover what influence the valency of a metal has upon the toxicity of its salts. In each of a series of experiments 1000 c.c. of 5/8 decinormal solution of sodium chloride in pure distilled water were used, with the addition of varying strengths of calcium sulphate. Grains of wheat, which previously had been soaked in distilled water, were placed in the solutions, and it was found that the stronger the calcium sulphate (up to 1/64 normal--the limit of experiment), the better the growth. The calcium sulphate was then replaced by salts of other bivalent metals, as zinc, lead and barium, with analogous results, the quantity necessary to obtain the maximum development varying with one and another; with zinc, n/128 gave the maximum. In this case the toxic action of both sodium chloride and zinc sulphate on germination were considerably reduced by their mutual presence--a result which fits in perfectly with what is known as to the masking effect of soluble substances upon toxic action. The same fact obtains in the animal kingdom, where Loeb and others have found that the toxicity of solutions of sodium chloride for marine animals is reduced by the introduction of salts of the bivalent metals.

"STIMULATION INDUCED BY ZINC COMPOUNDS.--While the toxic action of zinc on the higher plants is so obvious that it forced itself upon the attention of investigators at an early date, the question of possible stimulus is so much more subtle that it has only come into prominence during the last twelve years, during which time an extraordinary amount of experimental work has been done with regard to it. One investigator, Gustavson, was somewhat in advance of his time, for as long ago as 1881 he hinted at the possibility that zinc, aluminium and other substances might act as stimulants or rather as accelerators. He indicated that the role of certain mineral salts in the plant economy is to enter into combination with the existing organic compounds, the resulting product of the reaction aiding in the formation of yet other purely organic compounds which ordinarily require for their formation either a very high temperature or a long time--in other words, such a mineral salt acts as a kind of accelerator.

This work was apparently not followed up immediately, but it evidently contains the germ of the 'catalytic' hypothesis of which so much has been made during recent years.

This work dealing with zinc as a stimulant to plant growth has yielded such various and apparently contradictory results that the question cannot yet be regarded as settled--it is even still more or less uncertain whether zinc compounds act as stimulants, or whether they are merely indifferent at concentrations below the toxic doses...

"STIMULATION IN SAND CULTURES.--While Jensen denied stimulation in wheat grown in water cultures even when the solutions were as dilute as n/100,000 zinc sulphate, yet he found increase of growth with the same plant in artificial soil (quartz flour) to which much stronger solutions of zinc sulphate, from 5n/10,000 - n/10,000, had been added.

"INCREASED GROWTH IN SOIL.--Nakamura (1904) dealt with a few plants of agricultural importance, adding .01 gram anhydrous zinc sulphate to 2300 grams air-dried soil. The marked individuality in the response of the various plants to the poison is very striking. Allium showed signs of increased growth throughout; Pisum was apparently improved in the early stages of growth, but when the dry weights were taken at the end of the experiment no increase manifested itself in the weights of the plants treated with zinc;

with Hordeum the same quantity of zinc exercised a consistently injurious action. These results with peas and barley corroborate those obtained in the Rothamsted experiments with water cultures in that zinc sulphate proved to be less toxic to peas than to barley.

Kanda found that both peas and beans when grown in soil as pot cultures were improved by larger quantities of zinc sulphate than when they were treated as water cultures--a result in full accordance with current knowledge.

Wheat is evidently peculiarly sensitive to the effects of zinc compounds under differing conditions. Javillier (1908 c) pointed out that while wheat is very susceptible to the toxic action of zinc, yet it can benefit by the presence of sufficiently small quantities of the compounds of the metal. Rice is another cereal that is said to respond to the action of zinc sulphate, as Roxas, working in pot cultures with soil both with and without the addition of nutritive salts, obtained an acceleration of growth on the addition of m/1000 zinc sulphate, a quantity so remarkably great that it might be expected to act as a toxic rather than as a stimulant.

With phanerogams the zinc question is not only concerned with the effect of the metal upon germination, but also with its effect upon the later growth of the green plants, and on the physiological functions involving the construction of substances at the expense of mineral elements and the carbon dioxide of the air. Javillier holds that the indications are that zinc would prove to be profitable if applied to crops as a 'complementary' manure."

PRELIMINARY WORK (1926-27)

Since the results of the experiments conducted by various investigators have varied considerably with regard to the effect of certain chemical compounds on plants, it was considered desirable to repeat their technique with the hope of clarifying and adding to the mass of information available on the subject. With this in view, the writer proceeded (1) to test out several chemicals and (2) to determine the proper amount to apply in order to obtain the most practical control of weeds without injuring the tree seedlings. Three chemical compounds were used, namely zinc sulphate, zinc chloride, and copper sulphate. These were applied in quantities ranging from 4 to 12 grams per square foot of soil surface. The plots thus treated had previously been sown to weed and conifer seeds. Pigweed (Amaranthus spp.), Lamb's Quarters (Chenopodium album L.), and Fall Dandelion (Leontodon autumnalis L.), were sown on all plots. The conifers used (Picea abies (L.) Karst., Pinus sylvestris L., Pinus strobus L.) were sown separately but on plots of all treatments. Greenhouse plots were used during the winter of 1925-26 and forest nursery plots were used in the spring. The chief difference in treatment was that no weed seeds were sown in the nursery plots.

The greenhouse plots yielded confirmatory results but, due to poor control, no conclusive observations were obtained from the nursery plots. In general there was some evidence of approximate agreement with greenhouse results. The greenhouse plots produced evidence that copper sulphate and zinc chloride were violently toxic to weeds and conifers in all quantities applied. Zinc sulphate was less toxic and yielded evidence to show some variation in the toxic effect produced on weeds and conifers. In all treated plots there was a higher percentage of emergence and survival of conifers than of weeds. Zinc sulphate, applied in quantities of less than 5 grams per square foot, did not reduce weed growth materially but when applied in quantities of over 8 grams per square foot conifer growth was reduced. It appeared that most weed seedlings were killed by the 7-g

injury

It was recognizdd, at the end of the first year, that certain details in technique had been omitted. It thus seemed best to repeat the work under greenhouse conditions and, as far as possible, rectify the recognized deficiencies of the preliminary work.

METHODS AND PROCEDURE (1927-28)

Plots.--The work of the second year was carried on wholly under greenhouse conditions (average daily temperature of 60°F.). A bench was divided into 80 compartments, each of which was 18 inches long, 8 inches wide, and 6 inches deep (Figs.1 and 2). One-half of these plots were filled with silt from the forest nursery and the remainder were filled with fine sand from a pit nearby. The silt was taken from the upper 6 inches and the sand was obtained from a point about twenty feet below the surface soil of the pit.

Sterilization.--In order to kill any seeds that might have been present the soil was steam sterilized by the inverted pan method. A galvanized iron pan 4 by 8 feet and 6 inches deep was constructed for the purpose. The plots were steamed from 3 to 4 hours under pressure of 38 to 40 pounds. That this method proved satisfactory was shown by the fact that potato tubers and various seeds, which had previously been buried at various depths in the soil, were cooked to such an extent that they had softened and burst open.

Seeds used.--Since it had been definitely shown during the first year's investigations that such weeds as Amaranthus spp., Chenopodium album L., and Leontodon autumnalis L., were materially reduced by applications of zinc sulphate in quantities of 5 grams or more per square foot of soil, it was decided that a more resistant weed would be chosen for this experiment. Wahlenberg (7), Hartley (3), and others found members of the grass family to be more resistant to the action of chemicals than most other groups of weeds studied. Accordingly, Foxtail grass (Setaria glauca (L.) Beauv.), which is a troublesome weed in the forest nursery, was selected. Seeds were gathered in the fall and stored at room temperature until used for the experiment. Frequent germination tests were made.

The two conifers chosen for the experiment were Norway Pine (Pinus resinosa Ait.) and Norway Spruce (Picea abies (L.) Karst.). Both of these species are of commercial importance and are grown in the forest nursery.

Chemical used.--Of the three chemicals (copper sulphate, zinc chloride, and zinc sulphate) used the first year, the last named had given the best results under conditions of the experiment, and was, therefore, used in this experiment.

Seeding.--The seeds were planted February 5, 1928. In the sand series (Fig.1) the first row was planted to Norway Pine, the second to Norway Spruce, and the third to Foxtail grass. The next three rows were planted in the same order as the first three, and the last two rows were planted to Norway Pine and Foxtail grass, respectively. The silt plots were planted according to the same plan.

Two hundred seeds were sown in each plot. The conifer seeds were covered with about one-fourth of an inch of sand, and the weed seeds were covered to a depth of about three-sixteenths of an inch. Fine sand was used to cover all seeds.

Applying chemical.--Immediately after sowing the seed, the chemical was applied. Six grams of zinc sulphate ($ZnSO_4$ $7H_2O$) dissolved in 250 c.c. of water was applied to plot No.2, row No.1 of the sand series; seven grams was applied to plot No.3; eight grams to plot No.4; and nine grams to plot No.5. The chemical was added in the same order to plots 2 to 5 of every row of the sand and silt series. Plot No.1 in each row was retained as a check.

Care of plots.--Strips of burlap were placed over the plots to prevent excessive loss of moisture from the surface by evaporation. These strips were removed as soon as the seedlings began to emerge. The plots were watered whenever the soil surface showed signs of dryness.

Emergence plots.--The first three rows of plots across the bench in the sand and silt series were chosen for emergence records.

As the seedlings appeared above the surface of the soil (or emerged) they were pulled up, counted, and recorded (Figs.6 to 11, and Tables VII to XII).

Survival plots.--Rows 4, 5, and 6 of the sand series and 4a, 5a, and 6a of the silt series were retained for survival plots. They were watered and cared for throughout the experiment. On March 4, each survival plot received a 20 c.c. application of Pfeffer's complete nutrient solution, and on March 27 a second application of 40 c.c. was added.

Soon after the seedlings emerged a number of them "damped-off" on the treated plots. These seedlings were removed from the plots and recorded according to the date that "damping-off" took place (Figs.12 to 15, and Tables XIII to XVI).

At the end of the experiment, seedlings were dug up and the following data were recorded: length of top, length of tap-root (or longest root in the case of grass), length of the longest lateral root, and the number of lateral roots. This data appears in tables I to IV. Typical seedlings, selected from the survival plots of the sand series, were photographed to show the top and root development (See Figs.3 to 5).

Study plots.--Rows 7 and 8 of the sand plots and 7a and 8a of the silt plots were reserved for seedling examinations. These plots received applications of nutrient solution similar to those of the survival plots. Seedlings were dug up from time to time in order to determine the effect of the chemical on the roots.

Seedlings grown in soil extracts.--At the close of the experiment, soil extracts were made from the survival plots by leaching a suitable volume of soil with an equal volume of water. Seedlings of Norway Spruce, Norway Pine, and Foxtail grass, which previously had been germinated in petri dishes, were transferred to 500 c.c. beakers containing the soil extracts, and allowed to grow for a few weeks, after which time an examination of the roots was made.

Germination of seeds soaked in chemical.--In order to determine whether seeds were killed before germination, the following test was made: Two hundred seeds each of Norway Spruce, Norway Pine, and Foxtail grass were soaked in a series of zinc sulphate solutions with concentrations ranging from $\frac{m}{4}$ to $\frac{m}{2048}$ for 24 hours, and then removed and germinated in petri dishes. As the seeds germinated they were counted and recorded. The percentage germination is recorded in Table V.

Hydrogen ion concentration.--At the end of the experiment p H readings were taken on samples of soil obtained from the treated and the untreated plots. This data appears in Table VI.

OBSERVATIONS AND RESULTS

Emergence in sand.--Figures 6 to 8 show the total number of seedlings, plotted in 5 day periods, which appeared above the surface of the soil. The difference in total numbers of Norway Pine and Norway Spruce seedlings which emerged in the check plots as compared with those which emerged in the treated plots appear not to be significant. The lines of Figs.6 and 7 have the same general trend and are close together, indicating that the chemical had little effect or influence on emergence of the conifers. The effect of the chemical on the emergence of Foxtail grass, however, was different, as is shown in Fig.8. There was a distinct reduction in the number of seedlings that emerged in the treated plots, and this reduction was in direct proportion to the amount of chemical applied. In plots containing 9 grams of chemical the number of seedlings that emerged was only about half that in the checks.

Emergence in silt.--Figures 9 to 11 show the results obtained from the silt plots. In general they correspond with those obtained in sand plots, but the total number of seedlings (both tree and weed) which emerged was less than those in corresponding sand plots. The Norway Pine plots varied less than did either Foxtail grass or Norway Spruce.

Survival plots.--Upon microscopic examination sickle-shaped spores (typical of Fusarium spp.) were found upon the roots of all "damped-off" seedlings. Figures 12 to 15 show in general that "damping-off" increased in direct proportion to the amount of chemical applied. More "damping-off" occurred in the silt than in the sand plots.

Photographs (Figs.3 to 5) show the contrast in the top and root growth between typical seedlings selected from the sand plots.

By a study of the data in Table I it is seen that the tops of Foxtail grass were considerably shorter in the treated than in the untreated plots. Only a slight difference was shown in the conifers. The rather uniform height of conifers may be attributed to their normal habit of seedling development. The data in Table II shows that the length of tap roots (or longest root in case of Foxtail grass) of both weed and conifer seedlings was much reduced in the treated plots. The data in Tables III and IV show that the length and number of lateral roots were materially reduced in weeds and conifers grown on the treated plots. Again the reduction was in direct proportion to the quantity of chemical applied. The effect produced was more pronounced on conifers than on Foxtail grass.

Seedlings grown in soil extracts.--The roots of seedlings which were grown in soil extracts were long, light colored, and vigorous in the extracts from check plots, whereas the roots grown in the extracts from the most heavily treated plots were short, shriveled, dark colored, unhealthy in appearance, and showed evidences of disintegration. The roots grown in the extracts from plots containing 6 to 8 grams of chemical were intermediate in appearance.

Germination of seeds soaked in solutions of zinc sulphate.--At concentrations of $\frac{m}{4}$ only 9.4 per cent of the Norway Spruce germinated (Table V). For $\frac{m}{8}$ nearly three times as many seeds germinated as for $\frac{m}{4}$. For concentrations of $\frac{m}{16}$ to $\frac{m}{2048}$ the rise in germination per cent is more gradual. Apparently the chemical, in concentrations of less than $\frac{m}{512}$, had no effect on germination. At concentrations of $\frac{m}{4}$ to $\frac{m}{8}$ about half as many Norway Pine seeds germinated as at concentrations of $\frac{m}{16}$ to $\frac{m}{2048}$. Concentrations of less than $\frac{m}{16}$ seemed to have no effect upon germination.

The data show that about half of the viable seeds of Foxtail grass were killed by a concentration of $\frac{m}{4}$. At $\frac{m}{8}$ only about 10 per cent of the seeds failed to germinate, and at concentrations of less than $\frac{m}{8}$ the seeds apparently were not injured.

Hydrogen ion concentration.--Table VI shows the p H readings which were recorded at the end of the experiment. The chemical treatment tended to increase the soil acidity. In the sand plots this increase amounted to about 1 p H unit, while in the silt plots the increase was less than one half of a p H unit. Light applications of the chemical seemed to have induced as much change in hydrogen ion concentration as the heavier applications.

DISCUSSION

It has been pointed out that the germination and growth of seeds such as Pigweed (Amaranthus spp.), Lamb's Quarters (Chenopodium album L.), and Fall Dandelion (Leontodon autumnalis L.), which are common weeds in the Forest nursery, were reduced by applications of zinc sulphate in quantities of chemical in excess of 5 grams per square foot of soil. It was found that when quantities of chemical in excess of 8 grams per square foot were added to the soil the roots of the conifers were injured. When the surface was permitted to become dry, it was found that the chemical concentration increased locally, resulting in the girdling or "corroding" of the young stems and roots. Frequent watering, however, eliminated this danger of killing the seedlings due to cumulative effects of the chemical.

The data in Table V indicate that zinc sulphate in concentrations of $\frac{m}{8}$ or greater kills seeds before germination. Nine grams of zinc sulphate (crystalline) dissolved in 250 c.c. of water gives a concentration of approximately $\frac{m}{8}$. The question then arises--why were not most of the seeds in all plots containing 9 grams of chemical killed? That they were not killed is shown by Figs.6 to 11 and Tables VII to XII. Probable explanations are: adsorption of the chemicals by the soil, formation of new chemical compounds with the soil rendering the zinc unavailable, and leaching. Another factor which might have accounted for the difference in germination per cent between the soaked and unsoaked seeds is the degree of aeration to which the seeds were subjected.

Hartley (3) has shown that the concentration of the chemical added to the soil is not as important as the quantity added per unit of surface. He stated that it makes little difference whether the chemical is dissolved in one volume of solvent or in three volumes. This apparently holds when applied to seeds in soil but not when applied to seeds in solutions. For example, in Table V is shown the difference in per cent of seeds (which had previously been soaked in $\frac{m}{8}$ and $\frac{m}{4}$ zinc sulphate solution) that germinated. This difference in per cent appears to be significant. Thus, in the case of Norway Spruce, the percentage germination of seeds which had been soaked in $\frac{m}{8}$ zinc sulphate solution was nearly three times that of seeds which had been soaked in $\frac{m}{4}$ zinc sulphate solution. The presence of "damping-off" fungi and the killing of plants occurred only in the treated plots. From an examination of Figs.12 to 15 and Tables XIII to XVI, it is apparent that the percentage

not in direct ratio

loss of plants is in direct ratio to the quantity of chemical used. This is in direct opposition to the results of other investigators who find that zinc sulphate inhibits the growth of "damping-off" organisms. But the presence of the fungus in all "damped-off" seedling plots is not conclusive proof that it was wholly responsible for the death of the seedlings. It is probable that the species of Fusarium which attacked the seedlings was a weak parasite and consequently found little resistance in the weakened seedlings. In a study of seedlings from the survival plots, on which 8 to 9 grams of zinc sulphate had been added, it was evident that the seedlings were unquestionably weakened. It is believed that this weakened condition of the seedlings was due to the chemical. Another possibility is that the fungus might have thrived better in the heavily treated plots, due to a change in soil acidity

Data in Table VI show that the acidity of the soil was greater in the treated than in the untreated plots.

No method of determining the actual number of seedlings killed by the chemical was found. However, it is shown in Figs.6, 7, 9, and 10, that in general the number of conifer seedlings which emerged was not reduced by the application of the chemical used. On the other hand, it is shown in Figs.8 and 11 that the emergence of the grass was materially reduced. It is thus apparent that the death of the conifers occurred after emergence.

An examination of the data in Tables I and IV shows that the seedlings in the treated plots did not develop the same as those in the check plots. The data show that the length and number of lateral roots, and the length of tap roots (or longest root in the case of grass) was reduced by applications of zinc sulphate. The differences in lengths of tops between seedlings of treated and untreated plots is outstanding in the case of the grass but is not so evident in the conifers. This lack of variation in top length of conifers would be expected because of their manner of growth. Practically all the top growth attained by conifer seedlings during the first year is attained during the early stages of growth shortly after emergence. Although the heights of the conifers varied little it was found that the stems and cotyledons were often distorted in the heavily treated plots.

In the Foxtail grass the effect of the chemical was apparent, in most cases, in that it caused the basal leaves to turn red and finally die. The failure of the seedlings to remain green and vigorous cannot be attributed to a lack of plant food because a nutrient solution had been added. Furthermore, the plants in the check plots of both sand and silt remained vigorous and produced a good crop of seeds. On the 6-gram plots some stunted plants, which survived the chemical treatment, produced a few seeds. On the heavily treated plots the grass plots survived for a time but sooner or later died.

Tables I to IV show clearly the results produced on all plots. It might be said, however, that many conifer seedlings on the 8- and 9-gram plots, whose roots were temporarily checked by the chemical treatment, later produced sturdy roots and growth was resumed. This rather abnormal growth resulted in the production of fewer and shorter lateral roots and a shorter tap root than in the checks. It explains in part the variation found in the tables. From the viewpoint of actual injury to the seedlings, these conifers were not injured to the extent that they appear to have been from the data in the tables. Many of them, if transplanted, would be practically as good as those of the checks.

The theory of the action of zinc sulphate on plant cells will not be discussed here in detail. It is believed that zinc causes the death of cells by direct poisoning or toxicity. Reference is made to Brenchley's discussion of this phase of the subject which will be found in this paper in the review of literature.

For a more complete discussion of the plant cell and its relation to external conditions the reader is referred to Stiles' (6) text on Permeability. Other useful references on related literature not used directly in this paper are appended under the heading of Related Literature.

SUMMARY

1. Of three chemicals tested in the preliminary work, zinc sulphate proved to be most promising for further investigation.

2. Most weed seedlings, exclusive of the grasses, were readily killed by applications of 7 grams or more of zinc sulphate per square foot of soil.

3. Applications of 5 grams or more of zinc sulphate per square foot of soil materially reduced the number of seedlings of Foxtail grass that emerged, while applications of 9 grams or less per square foot apparently did not interfere with the emergence of Norway Spruce or Norway Pine seedlings.

4. Conifer seedlings (especially Norway Spruce) from the survival plots to which applications of 9 grams or more of zinc sulphate had been added were distinctly weakened by the chemical.

5. Seedlings grown in water cultures from soil extracts of the treated plots were injured in the same order as those grown in soil.

6. Interference by "damping-off" organisms introduced complications which made it difficult to determine the number of

seedlings killed by the chemical.

7. The soil was rendered more acid in the treated plots. This increase in acidity amounted to about a p H unit in the sand plots and less than one-half a p H unit in the silt plots.

8. The number of seedlings "damped-off" in the treated plots was in direct ratio to the amount of chemical applied. No "damping-off" of seedlings occurred in the check plots.

increased as more chemical was added

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Table I

Plant	Soil	Amount of zinc sulphate per square foot				
		Check	6gms.	7gms.	8gms.	9gms.
Foxtail grass	Sand	14.31	5.64	4.72	4.13	3.44
	Silt	22.55	7.29	5.21	8.34	7.83
Norway Spruce	Sand	2.45	2.45	2.27	2.35	2.04
	Silt	2.29	2.35	2.42	2.15	----
Norway Pine	Sand	3.22	3.24	3.13	3.25	2.28
	Silt	2.87	3.16	3.09	2.79	2.58

Length of top in centimeters. (Based on 100 measurements).

Table II

Plant	Soil	Amount of zinc sulphate per square foot				
		Check	6gms.	7gms.	8gms.	9gms.
Foxtail grass	Sand	14.42	8.26	5.34	4.74	4.75
	Silt	13.16	5.92	3.66	5.32	5.61
Norway Spruce	Sand	8.72	6.60	4.82	4.06	3.90
	Silt	7.60	6.74	7.10	4.84	----
Norway Pine	Sand	13.08	10.96	8.48	4.70	3.94
	Silt	9.38	7.96	6.20	3.36	3.80

Penetration of longest root in centimeters. (Based on 25 measurements).

Table III

Plant	Soil	Amount of zinc sulphate per square foot				
		Check	6gms.	7gms.	8gms.	9gms.
Foxtail grass	Sand	3.98	2.64	2.38	2.10	2.20
	Silt	3.66	2.38	1.52	1.78	1.56
Norway Spruce	Sand	4.12	3.18	2.96	3.46	1.80
	Silt	5.16	3.16	2.28	2.84	----
Norway Pine	Sand	7.16	4.74	4.74	2.38	1.88
	Silt	9.16	4.10	3.26	2.50	1.58

Length of longest lateral root in centimeters. (Based on 25 measurements).

Table IV

Plant	Soil	Amount of zinc sulphate per square foot				
		Check	6gms.	7gms.	8gms.	9gms.
Foxtail grass	Sand	6.40	4.60	4.84	4.24	3.80
	Silt	6.32	5.28	3.92	4.84	4.77
Norway Spruce	Sand	12.40	8.32	7.44	5.13	4.60
	Silt	6.64	9.88	9.52	5.69	2.25
Norway Pine	Sand	11.52	9.44	8.60	6.32	4.52
	Silt	7.88	7.80	6.56	5.48	5.12

Number of lateral roots. (Based on 25 measurements).

Table V

Plant	Concentration of zinc sulphate									
	$\frac{m}{4}$	$\frac{m}{8}$	$\frac{m}{16}$	$\frac{m}{32}$	$\frac{m}{64}$	$\frac{m}{128}$	$\frac{m}{256}$	$\frac{m}{512}$	$\frac{m}{1024}$	$\frac{m}{2048}$
Norway Spruce	9.4	26.9	42.5	45.9	50.0	57.2	59.3	66.7	68.7	68.8
Norway Pine	39.1	45.9	84.2	89.7	87.5	90.0	81.3	80.5	83.7	80.0
Foxtail grass	47.2	81.3	94.6	93.6	95.1	92.7	100	94.7	93.9	92.5

Germination in per cent of seeds which were soaked in solutions of zinc sulphate for 24 hours. (Based on 200 seeds).

Table VI

Soil	Zinc sulphate per square foot	Depth of sample	p H
Sand	Check	top	7.80
		bottom	7.25
	6 gms.	top	----
		bottom	----
	7 gms.	top	6.35
		bottom	6.25
	8 gms.	top	6.30
		bottom	6.35
	9 gms.	top	----
		bottom	----
	untreated	20 feet	7.25
Silt	Check	top	5.50
		bottom	5.35
	6 gms.	top	----
		bottom	----
	7 gms.	top	5.15
		bottom	5.10
	8 gms.	top	5.15
		bottom	5.10
	9 gms.	top	----
		bottom	----
	untreated near	surface	4.90
Nutrient solution			5.10

p H readings of the treated and untreated soil.

Table VII

Norway Spruce on sand					
		Zinc sulphate			
Date	Check	6gms.	7gms.	8gms.	9gms.
Feb.24	61	50	60	33	26
" 29	85	79	88	66	53
Mar. 5	96	94	106	92	77
" 10	102	96	115	100	93
" 15	102	96	117	102	93
" 20	102	97	119	103	97
" 25	104	103	123	106	101
" 30	106	104	129	109	106
Apr. 4	107	106	129	110	106
" 9	108	107	131	112	106
" 14	108		131	112	106
" 19	108		131	113	106
" 24	109		132		106
" 29	109				108
May 4	110				
Total	110	107	132	113	108

Table VIII

Norway Spruce on silt					
		Zinc sulphate			
Date	Check	6gms.	7gms.	8gms.	9gms.
Feb.25	33	16	2	2	2
Mar. 1	69	60	16	12	8
" 6	86	85	44	36	41
" 11	90	94	60	59	54
" 16	92	98	68	68	59
" 21	92	101	73	75	60
" 26	92	103	74	81	61
" 31	92	103	76	83	62
Apr. 5	93	103	78	84	64
" 10	93	104	80	85	64
" 15	93	104	80	85	64
" 20	93	105	80	85	64
" 25	93		82	85	64
" 30	94		83	87	66
Total	94	105	83	87	66

Total number of seedlings emerged.

Table IX

Norway Pine on sand					
		Zinc sulphate			
Date	Check	6gms.	7gms.	8gms.	9gms.
Feb.28	19	38	35	28	19
Mar. 4	78	79	82	68	69
" 9	106	96	102	105	107
" 14	116	104	111	113	118
" 19	127	112	117	118	124
" 24	135	120	127	127	133
" 29	145	132	140	131	138
Apr. 3	152	133	144	144	148
" 8	156	135	149	149	155
" 13	157	136	150	152	156
" 18				152	157
" 23				153	157
" 28					157
May 3					158
Total	157	136	150	153	158

Table X

Norway Pine on silt					
		Zinc sulphate			
Date	Check	6gms.	7gms.	8gms.	9gms.
Feb.29	17	14	2	11	28
Mar. 5	88	62	25	58	91
" 10	123	84	77	112	120
" 15	132	93	84	120	132
" 20	139	102	95	129	138
" 25	143	107	99	137	139
" 30	148	107	106	139	141
Apr. 4	150	109	109	145	142
" 9	153	113	114	147	143
" 14	153	114	115	150	145
" 19	157	117	119	154	148
" 24		118	123	155	148
" 29		119	130	156	149
May 4			131		150
Total	157	119	131	156	150

Total number of seedlings emerged.

Table XI

Foxtail grass on sand

Date	Check	Zinc sulphate 6gms.	7gms.	8gms.	9gms.
Feb.20	173	135	129	96	51
" 25	175	140	133	108	71
Mar. 1	177	142	135	112	84
" 6	179	142	135	113	92
" 11	179	142	137	115	96
" 16	179	143	138	116	97
" 21	179	144	139	116	97
" 26	180	145	140	116	97
Apr. 1				117	98
Total	180	145	140	117	98

Table XII

Foxtail grass on silt

Date	Check	Zinc sulphate 6gms.	7gms.	8gms.	9gms.
Feb.20	82	41	2	10	11
" 25	130	87	29	42	23
Mar. 1	143	102	45	59	31
" 6	152	107	80	72	32
" 11	152	114	103	81	35
" 16	156	115	109	84	37
" 21	156		113		
" 26	159		115		
" 31	160				
Total	160	115	115	84	37

Total number of seedlings emerged.

Table XIII

Norway Spruce on sand

Date	Check	Zinc sulphate 6gms.	7gms.	8gms.	9gms.
Mar.10					
" 11					
" 15		4			3
" 16					
" 17		7			
" 18					
" 19					
" 20		9	2		6
" 21					
" 22					
" 23					
" 24		10	6	4	12
" 25					
" 26		12		11	24
" 27					
" 28			7	12	26
" 29					
" 30					
" 31					30
Apr. 1					
" 2					31
" 3					
" 4					
" 5					
" 6			8	15	36
" 7					
" 8					
" 9					
" 10				17	42
" 11					
" 16					
" 17					44
Total	0	12	8	17	44

Table XIV

Norway Spruce on silt

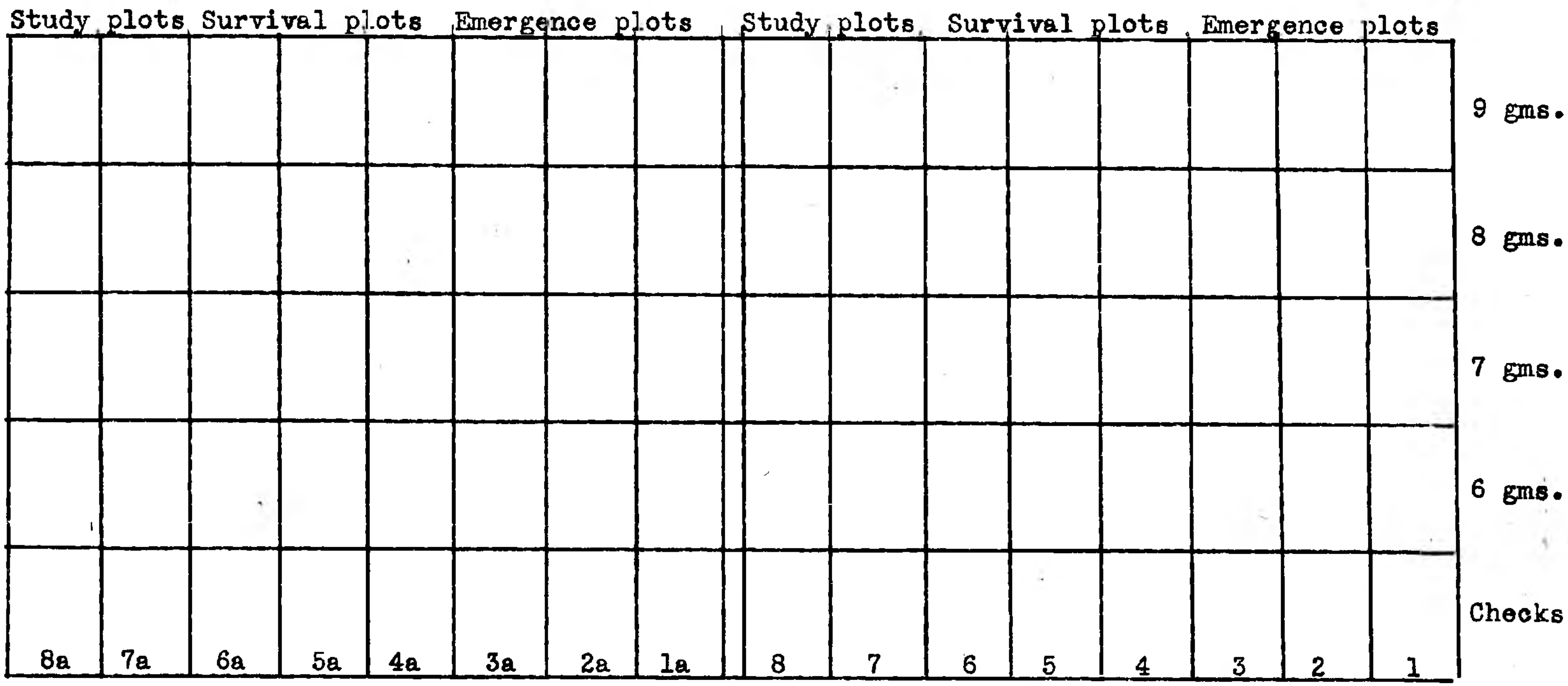
Date	Check	Zinc sulphate 6gms.	7gms.	8gms.	9gms.
Mar.10		3			
" 11					
" 15		4		1	1
" 16					
" 17				6	
" 18					
" 19					
" 20		10	4	11	21
" 21					
" 22					
" 23					
" 24			22	25	38
" 25					
" 26			24	31	47
" 27					
" 28			29	35	57
" 29					
" 30					
" 31		11	30	41	59
Apr. 1					
" 2		12	33	47	66
" 3					
" 4					
" 5					
" 6		14	37	53	67
" 7					
" 8					
" 9					
" 10			41	56	71
" 11					
" 16					
" 17		16	42	58	73
Total	0	16	42	58	73

Total number of seedlings "Damped-off".

Table XV — Norway Pine on sand; Table XVI — Norway Pine on silt

Date	XV: Norway Pine on sand — Check	XV: Zinc sulphate 6gms.	XV: Zinc sulphate 7gms.	XV: Zinc sulphate 8gms.	XV: Zinc sulphate 9gms.	XVI: Norway Pine on silt — Check	XVI: Zinc sulphate 6gms.	XVI: Zinc sulphate 7gms.	XVI: Zinc sulphate 8gms.	XVI: Zinc sulphate 9gms.
Mar. 10				1						
" 11										
" 15		1		3	1		3			
" 16										
" 17			1	4	5		11			2
" 18										
" 19										
" 20		3			15		12		2	16
" 21										
" 22										
" 23										
" 24				6	25		16	5	6	52
" 25										
" 26				7	40			7	8	78
" 27										
" 28				8	41		18		10	94
" 29										
" 30										
" 31			4		42		20	10	14	95
Apr. 1										
" 2				16	43		21		16	97
" 3										
" 4										
" 5										
" 6		4	6	17	47				17	101
" 7										
" 8										
" 9										
" 10			8	18	49			14	18	105
" 11										
" 16										
" 17					50				20	106
Total	0	4	8	18	50	0	21	14	20	106

Total number of seedlings "Damped-off".



Silt

1 & 1a Norway Spruce

2 & 2a Norway Pine

3 & 3a Foxtail grass

4 & 4a Norway Spruce

Sand

5 & 5a Norway Pine

6 & 6a Foxtail grass

7 & 7a Norway Pine

8 & 8a Foxtail grass

Fig.1. Diagram of plots.

Fig.2. Picture of plots.

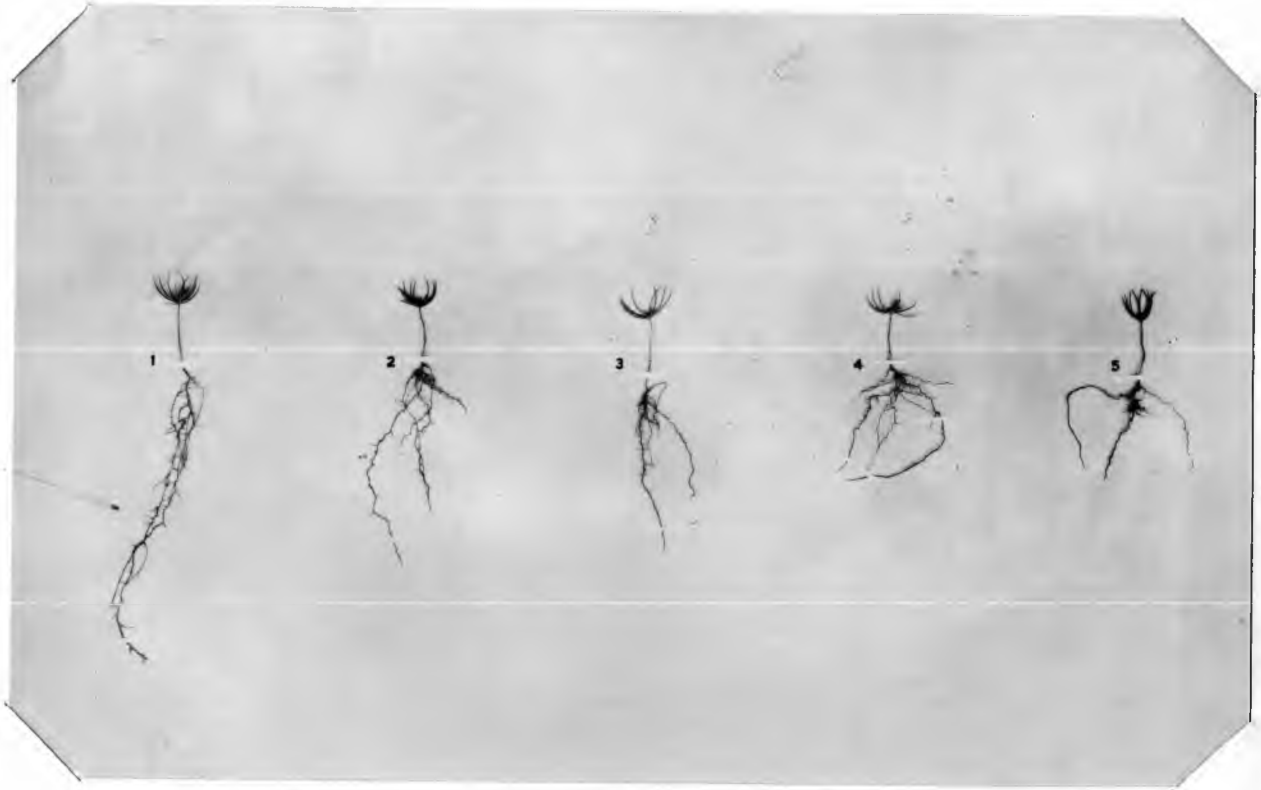


Fig.3. Norway Spruce in sand.

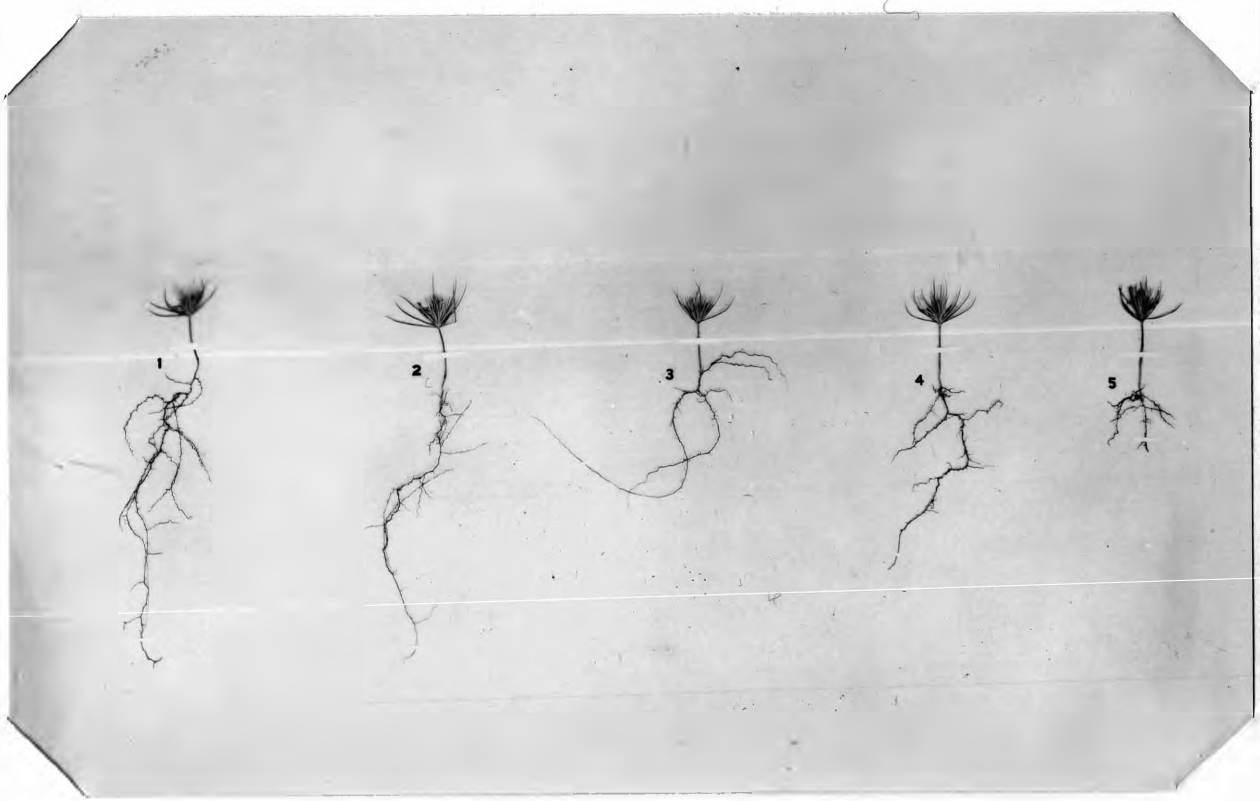


Fig.4. Norway Pine in sand.

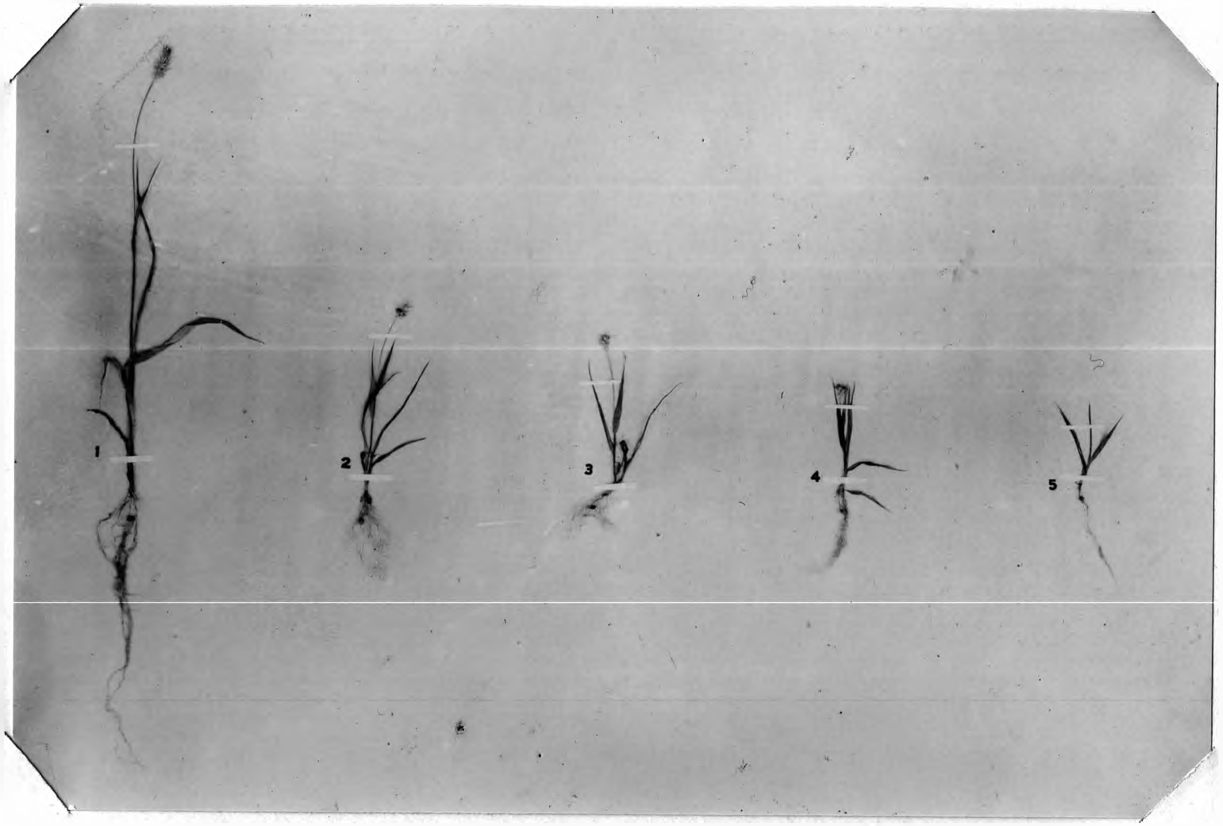


Fig.5. Foxtail grass in sand.

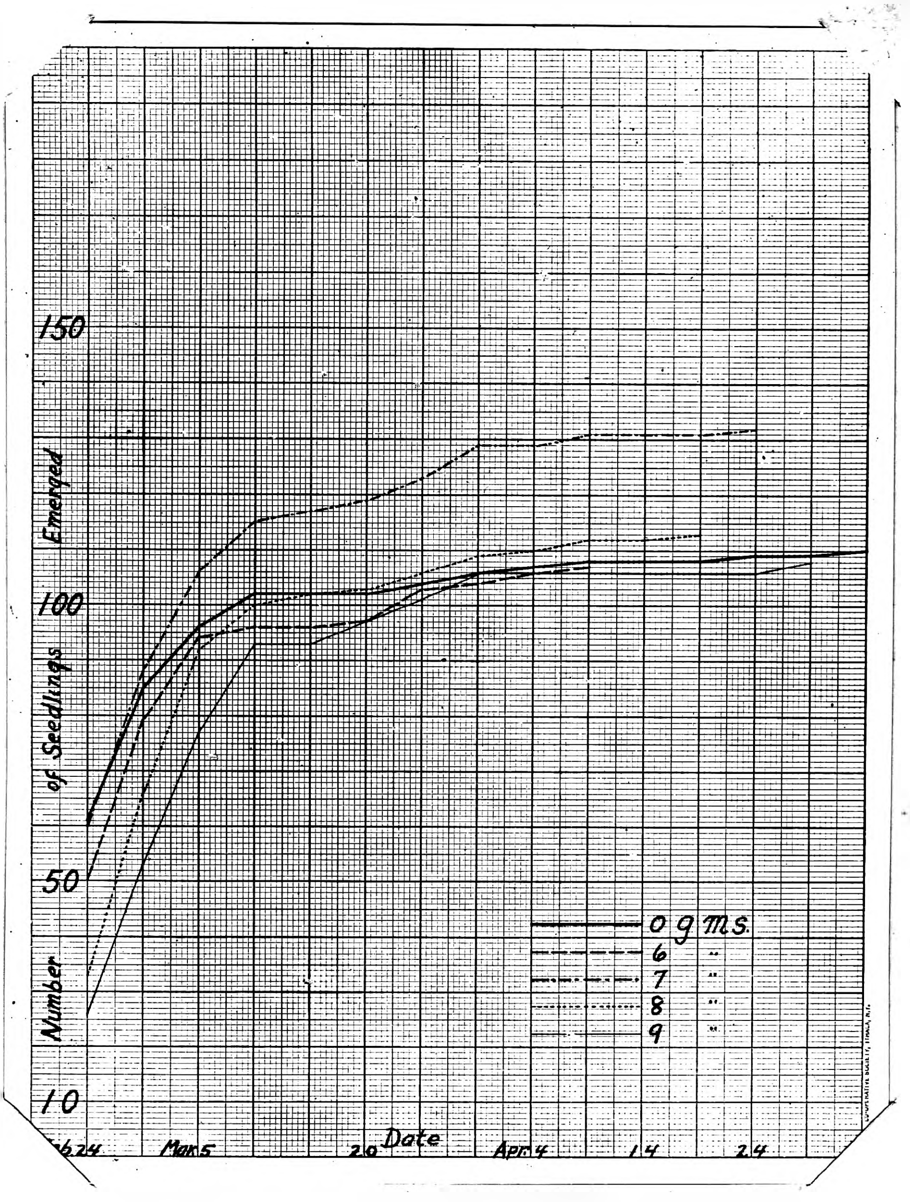


Fig.6. Norway Spruce in sand.

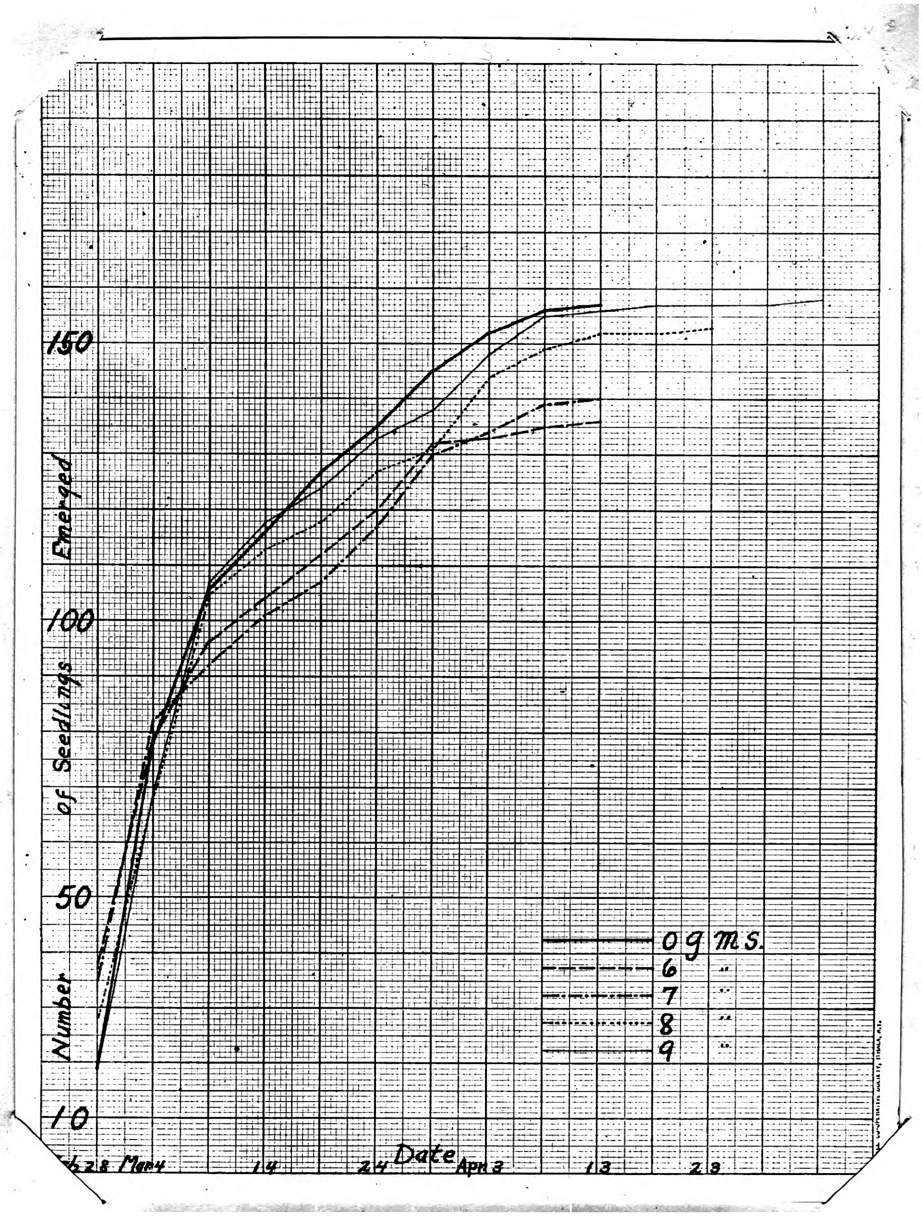


Fig.7. Norway Pine in sand.

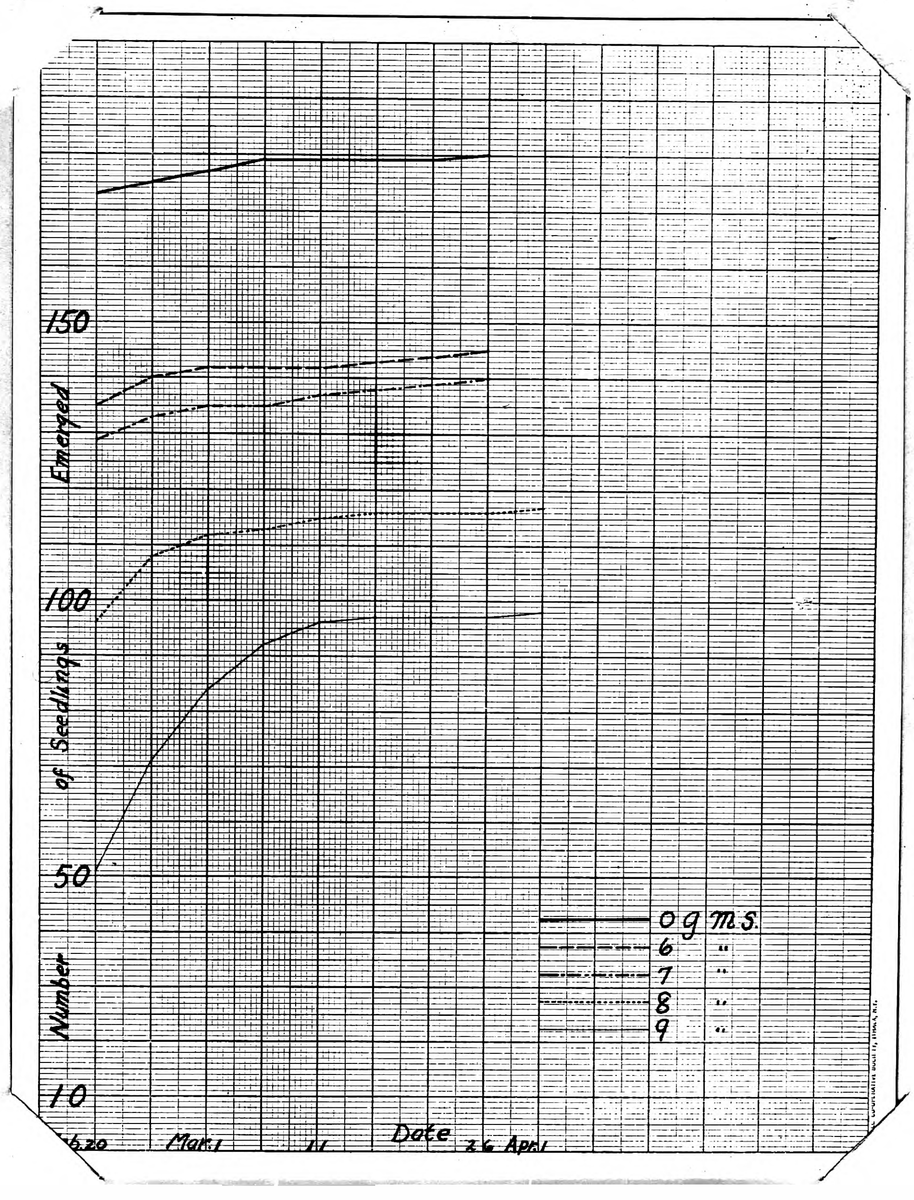


Fig.8. Foxtail grass in sand.

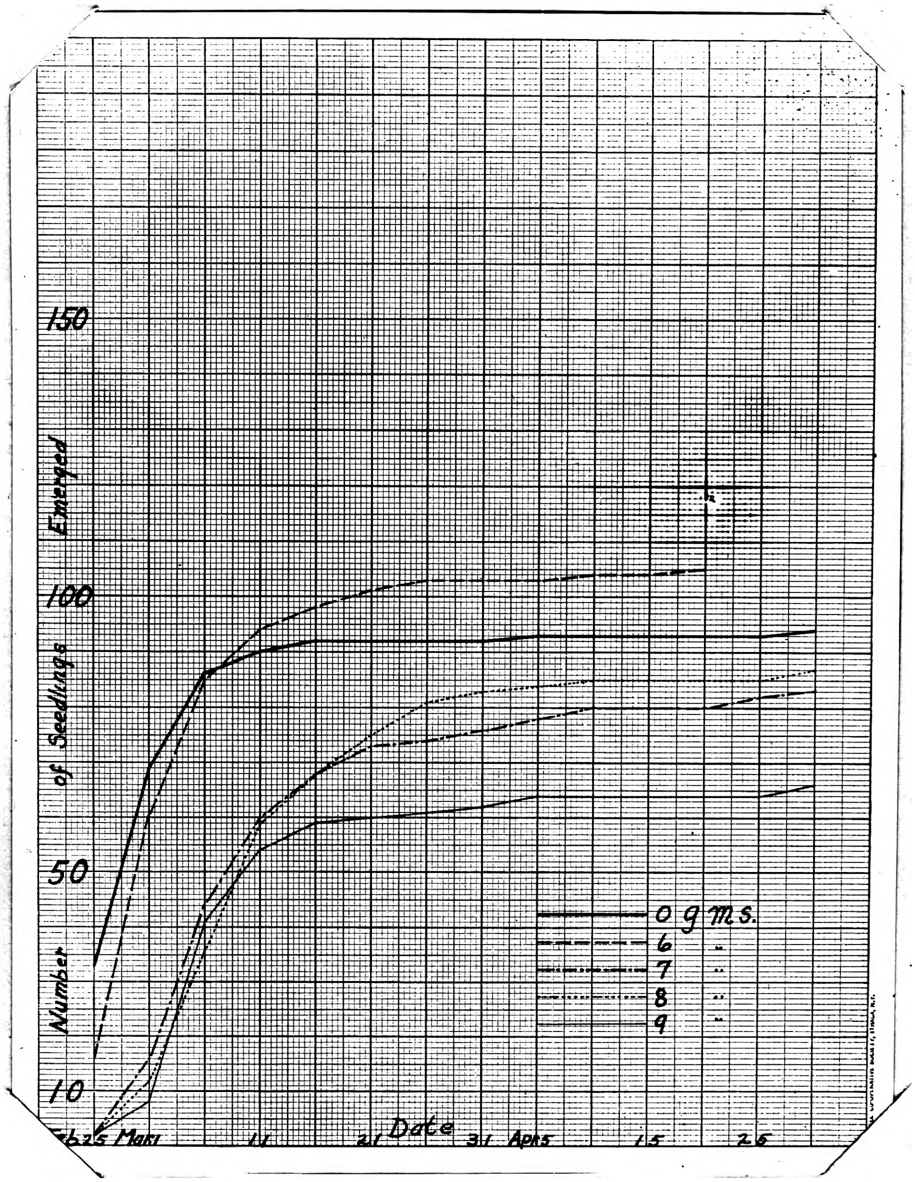


Fig.9. Norway Spruce in silt.

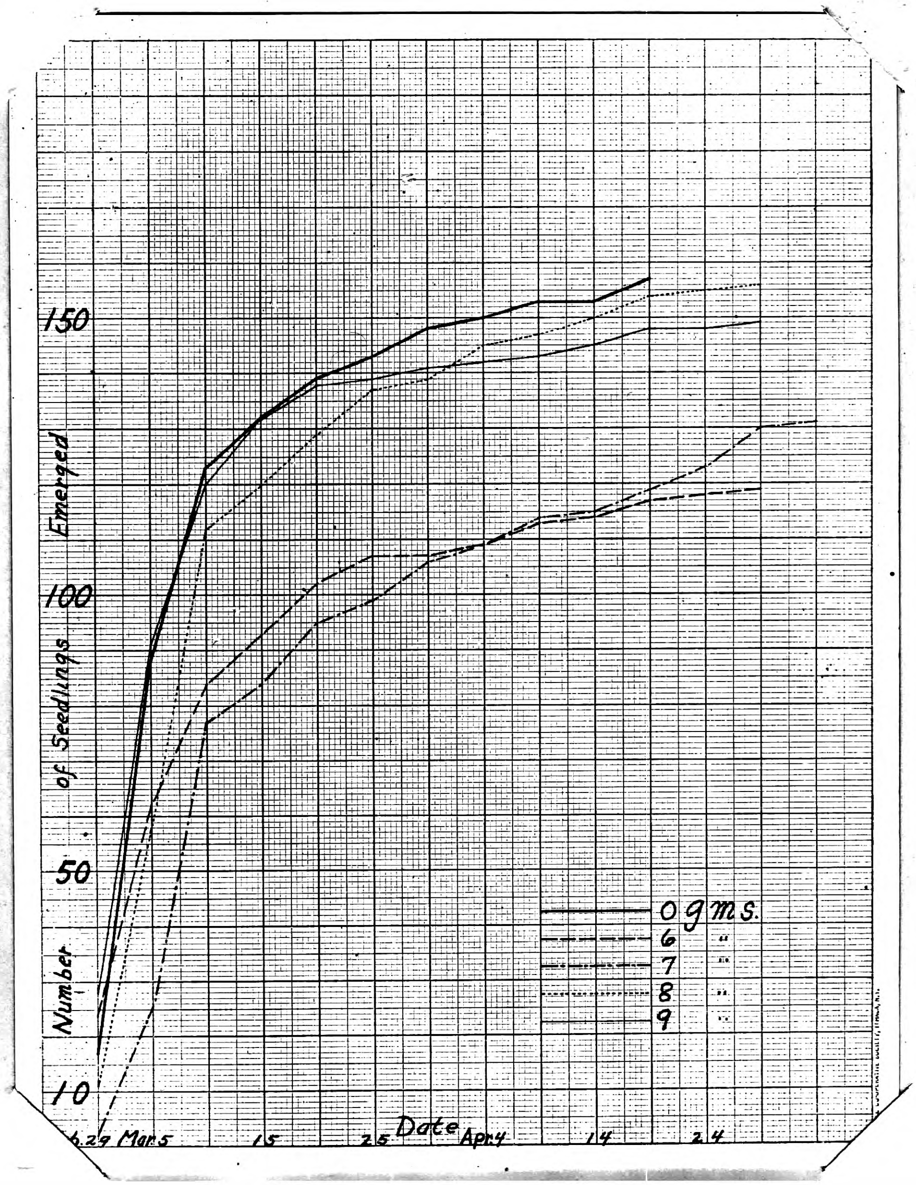


Fig.10. Norway Pine in silt.

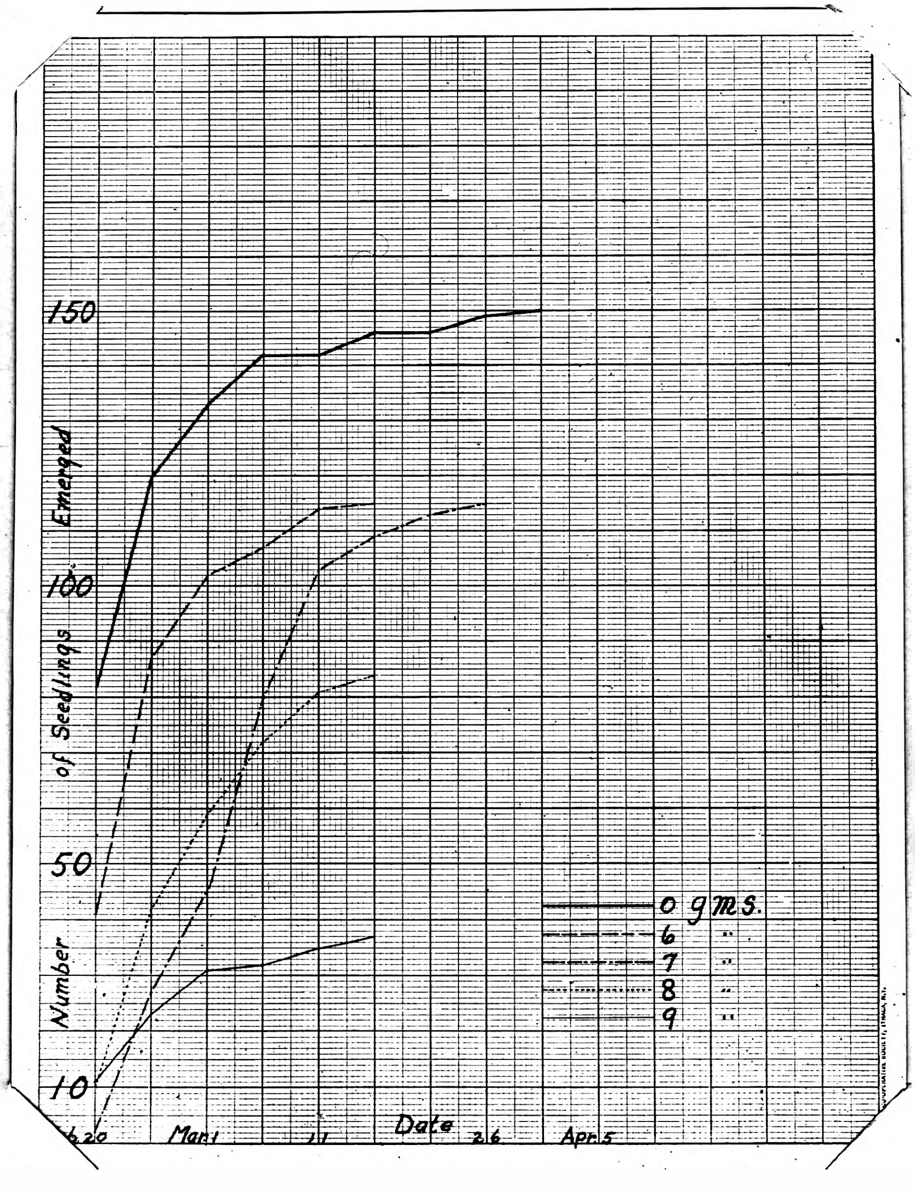


Fig.11. Foxtail grass in silt.

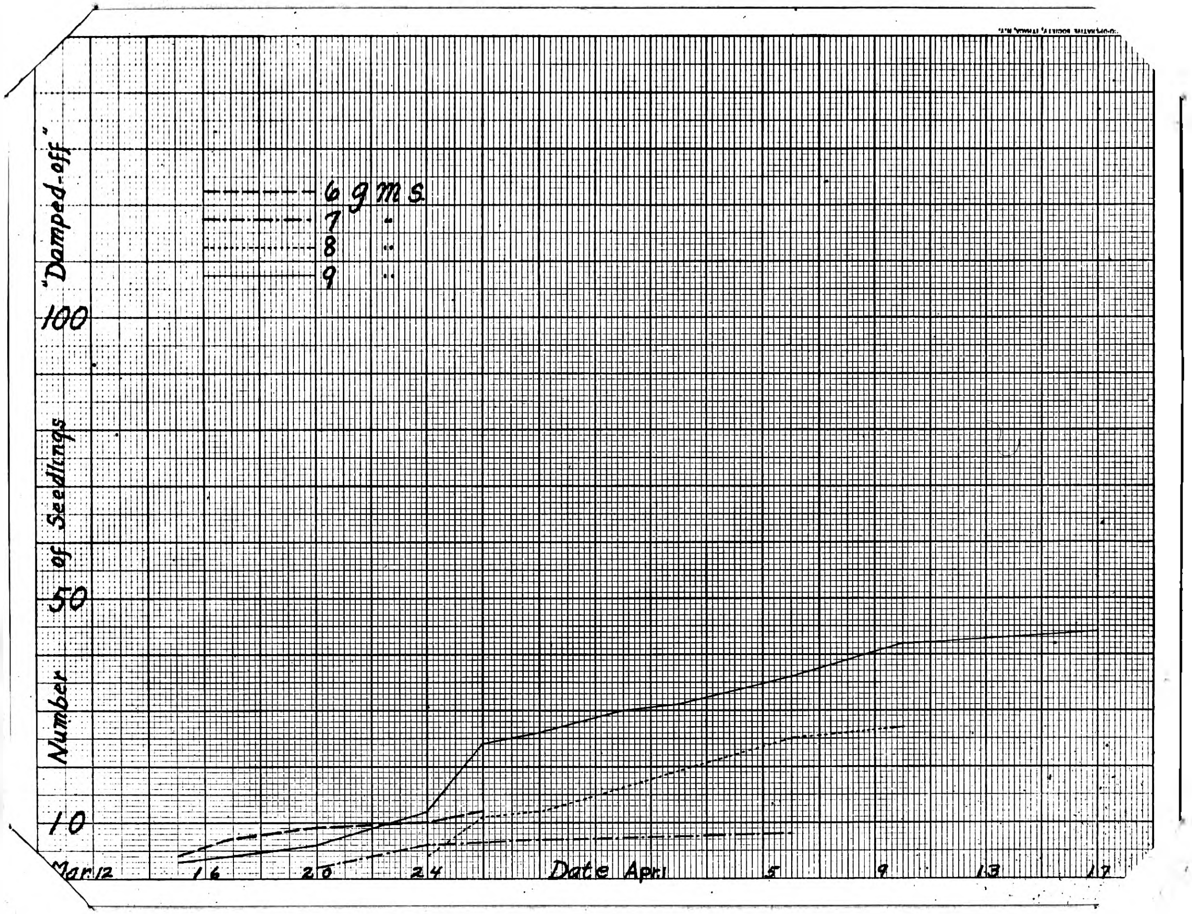


Fig.12. Norway Spruce in sand.

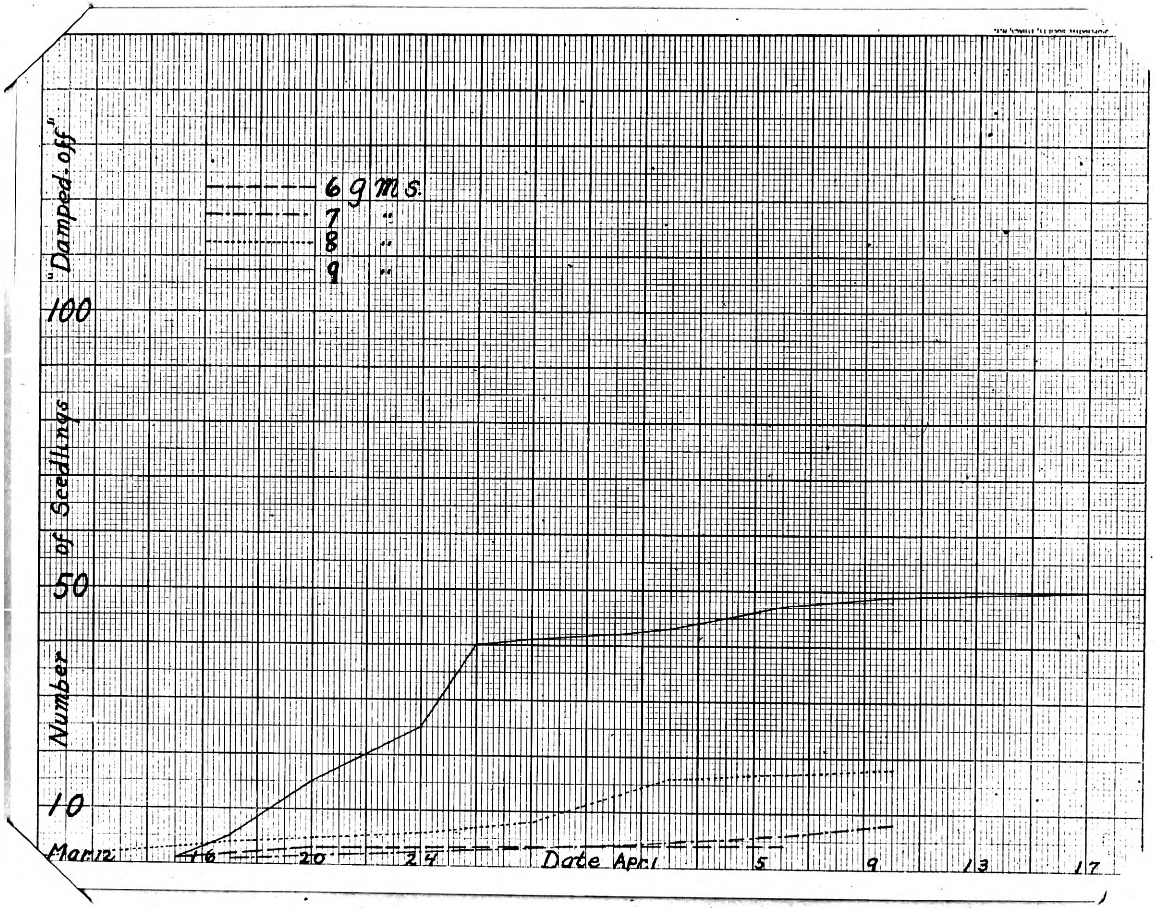


Fig.13. Norway Pine in sand.

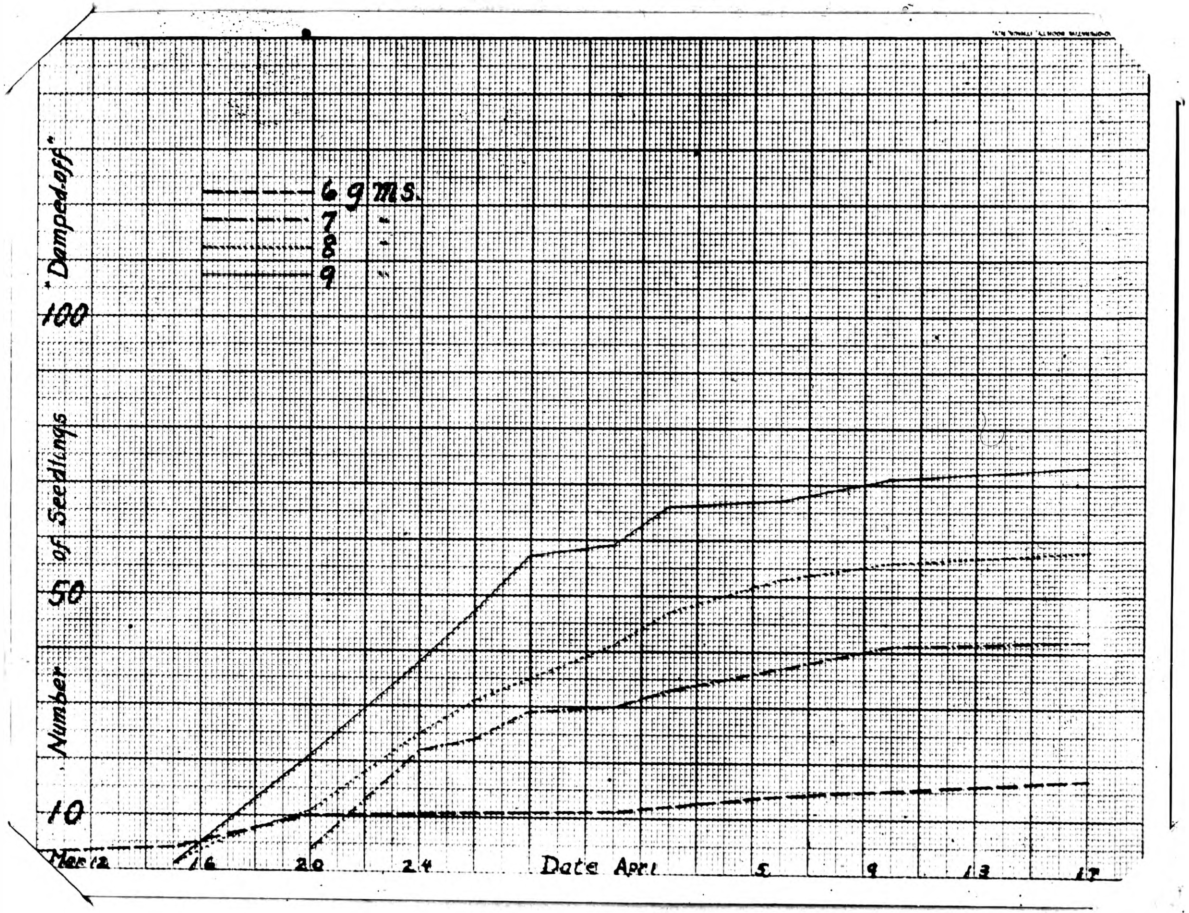


Fig.14. Norway Spruce in silt.

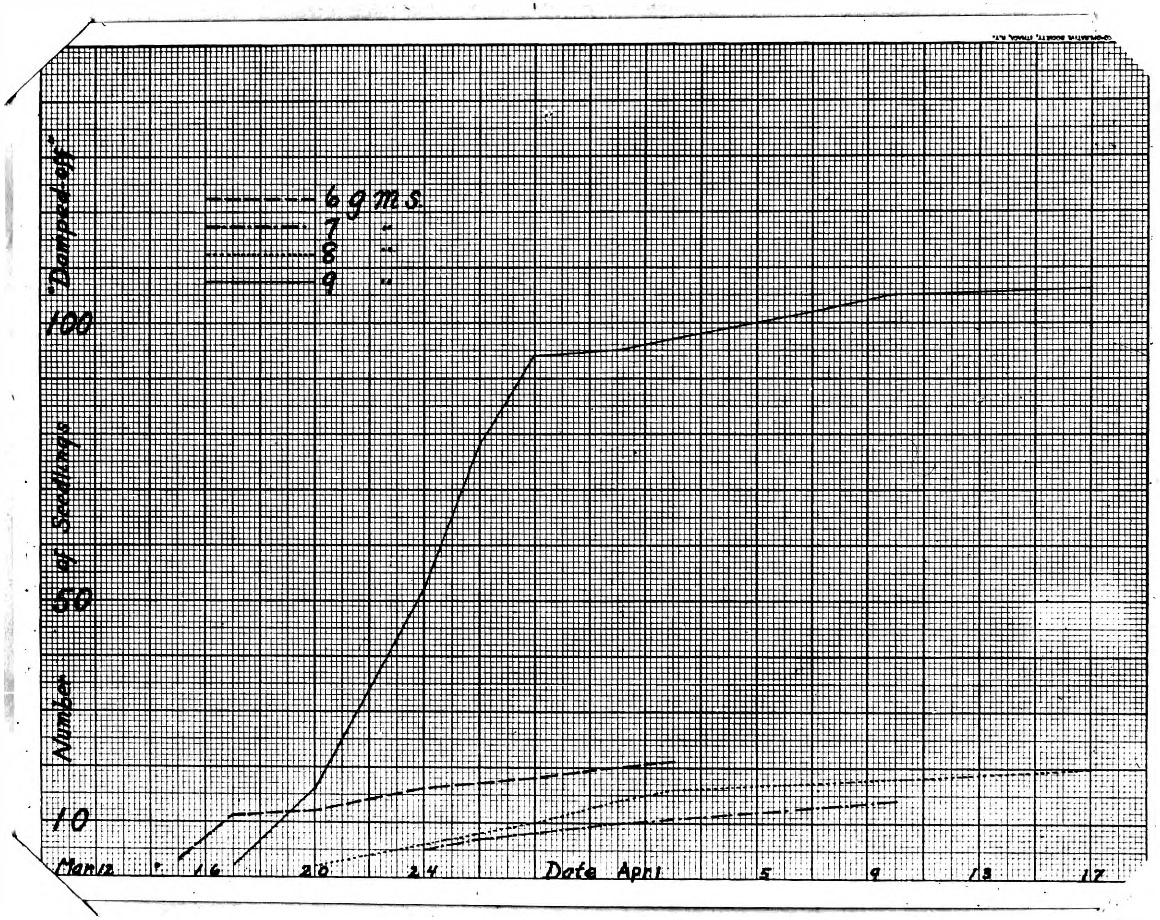


Fig.15. Norway Pine in silt.